

(51) International Patent Classification 6 : C12N 15/29, 15/54, C07K 14/415, C12N 9/12, A01H 5/00, A01N 65/00		A1	(11) International Publication Number: WO 99/36542
(21) International Application Number: PCT/SG98/00004 (22) International Filing Date: 16 January 1998 (16.01.98)		(43) International Publication Date: 22 July 1999 (22.07.99)	
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(54) Title: GENE ASSOCIATED WITH DISEASE RESISTANCE IN PLANTS			
(57) Abstract A gene encoding a novel mitogen-activated protein ("MAP") kinase has been identified in rice, isolated and cloned. Expression of the gene is induced in response to infection with the blast pathogen, <i>M.grisea</i> . The gene has utility in conferring disease resistance to plants, particularly monocotyledonous plants, such as rice, wheat, maize, barley and asparagus. Vectors containing the novel gene and transformed plant cells, plants and seeds are also disclosed.			

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GENE ASSOCIATED WITH DISEASE RESISTANCE IN PLANTS

Background of the Invention

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Field of the Invention

Bacterial, fungal and viral infections of plants grown for food and fiber cause substantial economic losses to farmers and consumers. For example, rice
10 blast is the most economically devastating disease of cultivated rice, caused by the filamentous fungus *Magnaporthe grisea* (Ou, 1985). (A bibliography is provided at the end of the written description.) It occurs in most rice growing areas worldwide, costs
15 farmers \$5 billion annually (Moffat, 1994). The disease reduces rice yield significantly, particularly in the temperate flooded and tropical upland rice ecosystem. The use of resistant cultivars is the most economical and effective method of controlling the
20 disease. With the advent of transgenic plant technology, it is possible to identify natural host defense mechanisms and to transfer genes associated with these mechanisms to or control expression of such genes in commercial cultivars. It is hoped that
25 expression of such genes will confer disease resistance to the transgenic plants.

Background Art

Over the last decades, much has been learned about the genetics of resistance to the blast fungus. While the molecular mechanism of host defenses to this pathogen is mostly unknown, blast fungus is believed to infect rice plants in a manner typical of other foliar pathogens. Infection by *M.grisea* is initiated when a conidium lands on a leaf surface. In a drop of water, a conidium produces a germ tube that grows and differentiates a specialized infection structure called an appressorium that adheres tightly to the plant surface (Bourett and Howard, 1990). The specialized cell generates enormous turgor pressure that is used to penetrate the underlying plant surface (Howard, 1994). The penetration into plant cells by pathogen invasion may damage the cell structure and activate genes responsive to wounding.

In plants, two mitogen-activated protein ("MAP") kinases involved in defense response to wounding have been identified (Usami et al., 1995; Bogre et al., 1997). Usami et al., (1995) reported a MAP kinase that is induced by wounding leaves from a variety of plant species including dicotyledonous and monocotyledonous plants. Another MAP kinase in alfalfa, p44MMK4, was activated by wounding. After wounding, the activity of p44MMK4 rose within 1 minute but decreased to basal levels within 30 minutes. It has been demonstrated that a MAP kinase, *PMK1*, plays a role in appressorium formation and infectious growth in rice blast fungus *M.grisea* (Xu and Hamer, 1996).

The MAP kinase signaling cascade is one of the major pathways involved in transducing extracellular stimuli into intracellular responses in mammals and yeasts (Shyy and Chien, 1997, Gabay et al., 1997, Samejima et al., 1997). MAP kinase is a specific class of serine/threonine protein kinases and has been implicated in a wide variety of physiological processes, such as cell growth, differentiation, oncogenesis and response to environmental stresses (Herskowitz, 1995, Cohen, 1997). In mammals, MAP kinases or extracellular signal regulated kinases ("ERKs") were originally identified as transducers of mitogens (substances that induce proliferation). Later, MAP kinases were also shown to be involved with signaling hormones, neurotransmitters and signals for differentiation (Marshall, 1994). At present, MAP kinase pathways are best understood in yeast and animals and several distinct MAP kinase pathways have been identified (Ruis and Schuller, 1995). The basic module of a MAP kinase cascade is a specific set of three functionally interlinked kinases. The activation of MAP kinases is brought about by upstream (i.e. earlier in the reaction sequence) kinases through phosphorylation of the conserved threonine and tyrosine residues that are located close to kinase domain VIII in all MAP kinases (Marshall, 1994; Hirt, 1997). These dual-specificity MAP kinase kinases (MAPKKs) can only catalyse the activation of specific MAP kinase and can not substitute for each other. The MAPKKs are themselves activated by phosphorylation through upstream kinases that either belong to the class of MAPKK kinases (MAKKs), or are raf and mos proteins (Marshall, 1994; Hirt, 1997).

In plants, several genes encoding MAP kinases have been identified from alfalfa (Jonak et al., 1993; 1995), *Arabidopsis* (Mizoguchi et al., 1994), pea (Stafstrom et al., 1993), petunia (Decroocq-Ferrant et al., 1995), tobacco (Wilson et al., 1993) and parsley (Ligterink et al., 1997). Similar to mammalian kinases, AtMAPK1 and AtMAPK2 are shown to be involved in cell proliferation (Jonak et al., 1993, Mizoguchi et al., 1994). Several stress-induced MAP kinases have also been identified in plants which are responsive to cold, heat, wounding, drought and mechanical stresses (Bogre et al., 1997, Jonak et al., 1996; Seo et al., 1995, Ligterink et al., 1997; Zhang and Klessig, 1997). The 48 kD MAP kinase, ERMK, is rapidly activated upon high-affinity binding of a fungal elicitor to a plasma membrane receptor in parsley cells (Ligterink et al., 1997). The activated ERMK is translocated into the nucleus where it may be involved in the transcriptional activation of defense genes. Recently, a MAP kinase, p48 SIP, is identified to be activated in tobacco cells by salicylic acid (SA) treatment which is an endogenous signal for the activation of several plant defense response (Zhang and Klessig, 1997).

These studies suggest that MAP kinases are an important component in the signal transduction pathway of plant defense to pathogen infection. Ligterink et al. (1997) and Zhang and Klessig, (1997) have found a elicitor-responsive MAP kinase in parsley suspension cells and a SA-activated MAP kinase in tobacco suspension cells respectively. However, no evidence was found that MAP kinase is activated by natural pathogen infection in plant species. Accordingly, a need exists for the identification of MAP kinase genes associated

with such defense mechanisms and means for expressing such genes in host plants (or regulating their expression) to confer disease resistance.

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Summary of the Invention

In accordance with this invention, a novel MAP kinase gene and protein that it encodes have been discovered. Based on sequence analysis, this novel
10 gene is a new member of the MAP kinase gene family which encodes a 519 amino acid 59 kD protein. It is designated as BIMK1 for blast induced MAP kinase. BIMK1 was strongly induced by rice blast fungus *M.grisea* and is postulated to be involved in the defense response of
15 rice to blast infection.

In one aspect, the invention relates to the deoxyribonucleic acid ("DNA") that comprises the novel MAP kinase gene, its messenger ribonucleic acid ("mRNA") transcript and the protein that it encodes. In related
20 aspects, the invention involves expression vectors that contain the novel gene operably linked to a plant active promoter and to plant cells and plants that have been transformed with such vectors.

In a further aspect, the invention concerns a
25 method for conferring disease resistance in plants, particularly monocot plants such as rice, wheat, maize, barley and asparagus, which comprises genetically modifying the plant to effect expression of the novel MAP kinase gene.

30

Brief Description of the Drawings

Figure 1 is an autoradiogram of a Southern hybridization analysis of restriction enzyme digested rice genomic DNA using labeled BIMK1 cDNA as a probe.

Figure 2 is an autoradiogram of a Northern analysis of total RNA (50 mg) isolated from rice leaf tissue at different time points after inoculation with *M.grisea* using labeled BIMK1 cDNA as a probe.

Detailed Description of the Invention

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The gene encoding a MAP kinase, identified as BIMK1 has been identified for rice, cloned and sequenced. The sequence of the full-length clone, including 5' and 3' untranslated regions, is provided in SEQ ID NO:1. The region from nucleotide 13 through nucleotide 1569 encodes the 519 amino acid 59kD protein whose sequence is shown in SEQ ID NO:2. The BIMK1 gene was isolated from rice infected with the rice blast pathogen, *Magnaporthe grisea*.

20

The invention provides an isolated DNA having substantially the sequence spanning nucleotides 13 through 1569 of SEQ ID NO:1. The invention further provides isolated mRNA complementary to the deoxyribonucleic acid having substantially the sequence spanning nucleotides 13 through 1569 of SEQ ID NO:1.

25

The invention also provides an isolated protein having substantially the sequence shown in SEQ ID NO:2.

"Isolated" as used herein, means that the nucleic acid or protein is in an environment different from its natural environment. For example, it may be cloned in a cloning or expression vector, it may reside in a bacterial cell, it may be associated with other means for transformation of plants or plant cells or it may

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reside in a plant with which it is not naturally associated. As used herein, the term "substantially the sequence" means a sequence that is predominantly that of the identified sequence, provided that the nucleic acid or protein retains the kinase functions of the native molecule. Thus, conservative substitutions, deletions and additions that do not significantly reduce the function of the protein are contemplated.

Probes, primers, antisense molecules and other nucleic acid molecules that are complementary to regions of the BIMK1 gene will be useful for its amplification and analysis, regulation of its expression and the like. Accordingly, the invention provides DNA or RNA molecules that are capable of hybridizing to the DNA molecules described above (or their complements) under stringent hybridization conditions. Such conditions are well known in the art and include those conditions under which stable hybrids will form when there is at least about 75%, preferably at least about 80%, most preferably at least about 90%-100% homology between the DNA or RNA molecule and the corresponding region of the target DNA.

The DNA can be incorporated in plant or bacterial cells using conventional recombinant DNA technologies. Generally, such techniques involve inserting the DNA into an expression vector which contains the necessary elements for the transcription and translation of the inserted protein coding sequences and one or more marker sequences to facilitate selection of transformed cells or plants.

A number of plant-active promoters are known in the art and may be used to effect expression of the nucleic acid sequences disclosed herein. Suitable

promoters include, for example, the nos promotor, the small subunit chlorophyll A/B binding polypeptide, the 35S promotor of cauliflower mosaic virus, and promoters naturally associated with MAP kinase genes, such as

5 BIMK1 in plants. SEQ ID NO: 6 provides the sequence of the 5' untranslated region upstream of the BIMK1 coding sequence. This region contains the putative promoter for this gene. SEQ ID NO: 6 overlaps the 5' end of the BIMK1 coding region, the ATG start codon appearing at

10 position 1378-80. A "TATA" box appears at positions 1302-1306 of the sequence. In addition to directing expression of the MAP kinase DNA described herein, this promoter has general utility as a plant-active promoter, particularly for effecting expression of

15 transgenes in monocotyledonous plants, such as rice.

Once the isolated DNA of the present invention has been cloned into an expression vector, it may be introduced into a plant cell using conventional transformation procedures. The term "plant cell" is

20 intended to encompass any cell derived from a plant including undifferentiated tissues such as callus and suspension cultures, as well as plant seeds, pollen or plant embryos. Plant tissues suitable transformation include leaf tissues, root tissues, meristems,

25 protoplasts, hypocotyls, cotyledons, scutellum, shoot apex, root, immature embryo, pollen, and anther.

One technique for transforming plants is by contacting tissue of such plants with an inoculum of a bacterium transformed with a vector comprising DNA in

30 accordance with the present invention. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48

to 72 hours on regeneration medium without antibiotics at 25-28° C.

Bacteria from the genus *Agrobacterium* can be utilized advantageously to transform plant cells.

5 Suitable species of such bacteria include *Agrobacterium tumefaciens* and *Agrobacterium rhizogens*. *Agrobacterium tumefaciens* (e.g., strains LBA4404 or EHA105) is particularly useful due to its well-known ability to transform plants.

10 Another approach to transforming plant cells with the nucleic acid of this invention involves propelling inert or biologically active particles into plant cells. This technique is disclosed in U.S. Pat. Nos. 4,945,050, 5,036,006 and 5,100,792 all to Sanford et.
15 al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior
20 thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector comprising the isolated DNA of this invention. Biologically active particles (e.g., dried yeast cells, dried bacterium or a
25 bacteriophage, each containing DNA sought to be introduced) can also be propelled into a plant cell tissue.

Another method of transforming plant cells is the electroporation method. This method involves mixing
30 the protoplasts and the desired DNA and forming holes in the cell membranes by electric pulse so as to introduce the DNA into the cells, thereby transforming the cells. This method currently has high

reproducibility and various genes have been introduced into monocotyledons, especially rice plants by this method (Toriyama et. al., 1988, Shimamoto et al., 1989 and Rhodes et al., 1988).

5 Similar to the electroporation method is a method in which the desired gene and protoplasts are mixed and the mixture is treated with polyethylene glycol ("PEG"), thereby introducing the gene into the protoplasts. This method is different from the
10 electroporation method in that PEG is used instead of an electric pulse (Zhang W. et. al., 1988, Datta et al., 1990 and Christou et al., 1991).

Other methods include 1) culturing seeds or embryos with nucleic acids (Topfer R. et al., 1989,
15 Ledoux et al., 1974) 2) treatment of pollen tube, (Luo et al., 1988) 3) liposome method (Caboche, 1990 and Gad et al., 1990) and 4) the microinjection method (Neuhaus G. et al., 1987).

Known methods for regenerating plants from
20 transformed plant cells may be used in preparing transgenic plants of the present invention. Generally, explants, callus tissues or suspension cultures can be exposed to the appropriate chemical environment (e.g., cytokinin and auxin) so the newly grown cells can
25 differentiate and give rise to embryos which then regenerate into roots and shoots.

The isolated DNA of the present invention is believed to be useful in enhancing resistance to disease-causing pathogens in both monocotyledonous
30 plants ("monocots"), and dicotyledonous plants ("dicots"). It is preferred for use with commercially important monocots, such as rice, wheat, barley, maize and asparagus.

In plants in which the BIMK1 gene naturally resides, enhanced disease resistance may be achieved by controlling expression of the endogenous gene, rather than transforming the plant with a vector containing the gene. Such control may be achieved, for example, by modifying or replacing endogenous promoters, enhancers or other control signals that regulate expression of the gene, for example, to achieve enhanced expression or programmed expression.

The predicted protein sequence of BIMK1 carries all 11 conserved domains for the catalytic function of serine/threonine protein kinase. The expression of BIMK1 was rapidly induced as early as 4 hours after inoculation with *M.grisea*, evincing the involvement of BIMK1 in the defense response to the blast fungus.

Several stress-induced MAP kinases have been identified in dicots. As shown in Table 1 below, the protein sequences of these genes showed 70-75% homology. For example, Parsley ERMK and tobacco SIMK have 74.4% protein identity. However, as shown in Table 1, BIMK1 only has about 50% identity with these two stress-related MAP kinases isolated from dicot plants. This suggests the divergence of MAP kinases in monocot and dicot plant species. In addition to sequence differences, BIMK1 is about 500 bp longer than all cloned MAP kinase genes. The 3' region of the gene contains a domain similar with ADH genes in animals. The function of this domain in the defense response to blast infection is unknown.

The invention is further illustrated by the following examples, which are not intended to be limiting.

EXAMPLES

Materials and Methods5 Rice plants and blast inoculation

The resistant isogenic line Cl01A51 carrying the Pi-2 gene and the susceptible cultivar C039 were used in the experiment. Three week-old rice plants were inoculated with a Philippine isolate PO6-6 of *M.grisea*.
10 After inoculation, plants were kept in dark in a dew chamber for 24 hours at 26° C. Then, inoculated plants were move into a growth chamber in 10 hours light with 14 hours dark at 25-26° C for 7 days. Leaf tissue was harvested from both cultivars at 0, 4, 8, 12, 24, 48.
15 72 hours after inoculation.

RNA isolation, cDNA svnthesis and RT-PCR

RNeasy mini kit (Qiagen, Germeny) was used to isolate total RNA from 150-200 mg rice leaf tissue.
20 Poly(A)+ RNA fractionated from total RNA using Qiagen Oligotex Spin Column was used as a template in a reverse transcriptase-mediated polymerase chain reaction (RT-PCR). Two primers, CF9-RT and CF9-Rev, were designed based on the DNA sequence of the cloned
25 gene Cf-9, a tomato resistance gene to the leaf mould fungus *Cladosporium fulvum* (Jones et al., 1994). The primer sequence of CF9-RT is 5'-AAAAGCACAAGTTGCTGC-3' (SEQ ID NO:3) which is the DNA sequence 217-235 bp after the start codon. The sequence of CF9-Rev is
30 5'TAACGTCTATCGACTTCT-3' (SEQ. ID NO:4) which is the reverse strand sequence of Cf-9 from 1408 to 1426 bp after the start codon. RT-PCR was conducted following protocols provided by the manufacturer (GIBCO-BRL,

Life-Technology, USA). The amplified cDNAs were then separated in 1.2% agarose gel.

Cloning and DNA sequencing

5 Specific bands were cloned into pGEM-T vector (Promega, USA). Clones were sequenced using the ABI PRISM 377 DNA sequencer (Perkin-Elmer, CA, USA). The sequence was analyzed with softwares DNASTar and Sequencher 3.0.

10

BAC library screening and subcloning

 Protocols for BAC filter preparation and screening were as described Wang et al. (1995). Hybridization and washing conditions were the same as described in
15 Hoheisel et al., (1993).

Southern hybridization

 Rice genomic DNA was isolated as described by Dellporta et al. (1984). DNA was digested with
20 restriction enzymes and separated in 0.8% agarose gel, and then transferred onto Hybond-N+ membrane (Amersham, UK). Probes were labeled using megaprimer labelling kit (Amersham, UK). Rapid hybridization solution (Clonetech, USA) was used.

25

Northern hybridization

Total RNA used in the Northern blot analysis was isolated using a Trizol total RNA isolation reagent (GIBCO-BRL, Life-Technology, USA). Fifty micrograms of total RNA per lane was separated in 1.0% agarose gel and transferred onto Hybond-N+ membrane (Amersham, UK) using NorthernMax kit (Ambion, USA) following the manufacturer's instruction. Northern hybridization was carried out same as Southern hybridization described above.

Example 1

Isolation of a cDNA fragment induced after blast infection

Total RNA was isolated from leaf tissue inoculated with isolate PO6-6 8 hours after inoculation. Purified mRNA was used as template in the first strand cDNA synthesis. When primers CF9-RT and CF9-Rev were used in RT-PCR, four bands were amplified in both ClO1A51 (compatible) and C039 (incompatible) post-inoculation (data not shown). These cDNA fragments were then cloned into the pGEM-T vector. Clones with different insert sizes were sequenced. A database search revealed that the clone with 350 bp insert is highly homologous to mammalian and yeast MAP kinases.

Example 2

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Isolation of genomic clones from rice BAC library

To clone the full-length genomic fragment of this gene, a rice BAC library of cultivar IR64 (Yang et al., 1997) was screened using the 350 bp cDNA fragment

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described in Example 1 as a probe. Four positive BAC clones (3-07, 17-H21, 43-H15 and 43-F5) were identified from the whole BAC library. The miniprepared DNA of the three BAC clones was digested with 3 different enzymes to check if they are overlapping clones in a chromosomal region. Based on the restriction patterns, it was found that these three clones were overlapping clones. Thus, one BAC clone (3-07) was chosen and subcloned into pBluescript-SK (Strategene, USA). The recombinant clone which hybridized with the 350 bp cDNA fragment (M1, 4.5 kb) was identified and used for sequencing. Based on a comparison with known MAP kinase genes, it was found that it contains the 5' region of the gene including the putative promoter and part of coding region (about 400 bp).

Example 3

Isolation of a full-length cDNA using RT-PCR

To isolate a full length cDNA from rice, a primer containing sequence spanning the start codon ATG(5'-AACACAGTGGAAATGGAGTTCTTCA-3') SEQ ID NO:5 was designed based on the genomic DNA sequence. RT-PCR was performed using this primer and a oligo-dT primer (Life-Technologies, USA). From the cDNA prepared from the infected leaves of Cl01A51 (8 hours after inoculation), a 2.0 kb PCR product was obtained. This PCR product was cloned into pGEM-T vector and sequenced. The sequence is shown in SEQ ID NO:1. It contains a 1557 bp open reading frame corresponding to 519 amino acids (SEQ ID NO:2). This gene was designated BIMK1 for blast induced MAP kinase. This amino acid sequence was compared to the sequence of

several MAP kinases isolated from a variety of organisms. As shown in Table 1, the sequences are significantly homologous. In section A of the Table, multiple alignment of the deduced amino acid sequence (N-terminal) of BIMK1 with other members of MAP kinases from other organisms is shown. The amino acid sequence of BIMK1 is compared to that of MsERK (Duerr et al., 1993) from *Medicago sativa*, WIPK (Seo et al., 1995) from tobacco, ATMPK (Mizuguchi et al., 1994) from *Arabidopsis*, ERK2 (Owaki et al., 1992) from human, ERM (Ligterink et al., 1997) from parsley. Bold type represents amino acid residues that match the EIMK1. Gaps were induced to maximize alignment. The conserved TXY (in BIMK1, "X" is an aspartic acid while in most MAP kinase it is a glutamic acid) phosphorylation motif for MAP kinase is indicated by asterix. The 11 MAP kinase subdomains are labeled in Roman numerals (Hanks et al., 1988). The *M.grisea* BIMK1 gene contains all 11 highly conserved subdomains which are present in all known MAP kinases in mammals and plants. Interestingly, BIMK1 also contains 50 amino acids homologous to mammalian alcohol dehydrogenase (ADH) in its C-terminal. Section B of the Table shows multiple alignment of the deduced amino acid sequence (C-terminal) of BIMK1 with other ADH genes in animals and plants. ADH is present in many organisms that metabolize ethanol, including human, in an oxidoreductase reaction with NAD⁺/NADH as an essential co-factor.

BIMK1 is conserved in rice genome and mapped to a region clustering blast resistance genes

DNAs of Cl01A51 and C039 were digested the restriction enzymes BamHI, EcoRI and HindIII. Southern hybridization was carried out as described in the section of Materials and Methods using the cDNA fragment of BIMK1 as probe. No polymorphism was detected between resistant and susceptible lines for three enzymes (Figure 1). Similar results have been obtained using DNAs of 4 other cultivars (data not shown). These result indicated that BIMK1 is conserved among rice cultivars. BIMK1 has been mapped on rice chromosome 12 between makers RG341 and RG574, a region clusterring rice blast resistance genes Pi-4(t) and Pi-6(t).

Example 5

BIMK1 was induced bv rice blast fungus

Total RNA was isolated from rice leaf tissue collected at different timepoints after inoculation. The blot was hybridized using BIMK1 cDNA fragment as probe labelled with 32p. It was found that BIMK1 was highly induced as early as 4 hours after inoculation. The expression of the gene BIMK1 was reduced 24 hours after inoculation (Figure 2). The induction level of BIMK1 in both resistant (Cl01A51) and susceptible (C039) lines was very similar (Figure 2). Since Cl01A51 and Co39 have the same genetic background except Cl01A51 carries a rice blast resistance gene, Pi-2, it is suggested that BIMK1 was induced independently from Pi-2 and is involved in a general defense pathway to blast.

Table 1

(A)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
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Table 1 (continued)

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48-kD kinase in tobacco. Plant Cell. 9(5): 809-824.

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15 76:835-840

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Institute for Molecular Agrobiolology (except for US)
He, Chaozu (for US)
Wang, Guo-Liang (for US)

(ii) TITLE OF INVENTION: Gene Associated with Disease Resistance in Plants

(iii) NUMBER OF SEQUENCES: 6

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1957 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: *Oryza sativa*
(B) STRAIN: C101A51

(vii) IMMEDIATE SOURCE:
(B) CLONE: BIMK1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AACACAGTGG AAATGGAGTT CTTCAGTGG TATGGAGAAG CAAGCCAGTA CCAGATCCAG	60
GAAGTCATTG GCAAAGGAAG TTATGGAGTA GTTGCTGCTG CAGTAGATAC CCGCACGGGT	120
GAGCGGGTTG CGATCAAGAA GATCAATGAT GTGTTTGAGC ATGTATCAGA CGCTACGCGC	180
ATATTGCGTG AGATCAAGCT CCTTCGTCTG CTCCGTCACC CAGACATAGC TGAGATCAAA	240
CACATTATGC TTCCCCCTTC TCGAAGGGAG TTCCAAGATA TTTATGTTGT TTTTGAGCTC	300
ATGGAATCAG ATCTCCATCA AGTCATCAGA GCGAACGATG ACCTCACCCC GGAGCACTAC	360
CAGTTTTTCC TGTACCAACT TCTTCGTGCT CTCAAGTACA TCCATGCAGC TAATGTATTT	420
CATCGCGATC TAAAGCCCAA GAATATACTG GCAAACCTCAG ACTGCAAATT GAAAATATGT	480
GATTTCCGAC TTGCCCCGAGC ATCATTCAAT GATGCCCTT CAGCAATATT TTGGACGGAT	540
TATGTTGCAA CGAGGTGGTA CCGAGCACCT GAATTATGTG GCTCATTTTT CTCCAAATAC	600
ACTCCTGCAA TTGATATTTG GAGTATTGGG TGCATATTTG CTGAACTTCT CACTGGGAGA	660
CCACTATTTT CTGGGAAGAA TGTGTGTCAC CAATTAGATA TTATAACAGA TCTTCTTGGA	720
ACTCCATCAT CAGAAACCTT ATCCAGGATT CGAAATGAGA AGGCCAGGAG ATACTTGAGC	780
ACCATGCGGA AAAAACATGC TGTCCCCTTC TCTCAGAAGT TCCGCAATAC TGACCCCTTG	840
GCTCTTCGTC TGCTAGAGCG TTTACTGGCA TTTGATCCTA AAGATCGGCC TTCAGCTGAA	900
GAAGCTTTGG CTGATCCGTA CTTGCAAGT CTTGCTAATG TGGAACGTGA GCCCTCAAGA	960
CATCCAATCT CAAAACCTGA GTTTGAATTC GAGAGACGGA AGCTGACAAA AGATGATGTT	1020

5 AGAGAATTAA TTTATCGAGA GATTTTGGAG TATCACCCAC AGATGCTGCA AGAGTATATG 1080
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 TTTGCACACC TTGAGGAGAA CTACAGCAAA GGAGAAAGAG GTTCTCCACT GCAGAGGAAG 1200
 CATGCTTCTT TACCGAGGGA GAGAGTAGGT GTATCAAAGG ATGGTTATAA CCAACAAAAC 1260
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 GCTCATCCAT GTTCACATAT TCTTCTTGCC ATTGTGCTGT CTGTCACTAC AGGACCCCTG 1740
 25 CATGGATTAA TGTATTATCC CTCTGATGTA AACTAGATT AGTTCATCTG TCCATGGAGG 1800
 AATGAATAGC AAGCAGCCAG CTTGTGCATC ATGTGGGCAT GTTCATTTTC CAGTGAGATC 1860
 30 TAGTCATATC CATGCTTTTT TTGTAATGGT ATATGAAACA GTTTATCAGT GAGACTGTGG 1920
 TCCATTCCTC TTTGAAGAAC TCCATTTCCA CTGTGTT 1957

(2) INFORMATION FOR SEQ ID NO:2:

35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 519 amino acids
 (B) TYPE: amino acid
 40 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

45 (iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

50 (A) ORGANISM: *Oryza sativa*
 (B) STRAIN: C101A51

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

55 Met Glu Phe Phe Thr Glu Tyr Gly Glu Ala Ser Gln Tyr Gln Ile Gln
 1 5 10 15
 60 Glu Val Ile Gly Lys Gly Ser Tyr Gly Val Val Ala Ala Ala Val Asp
 20 25 30
 Thr Arg Thr Gly Glu Arg Val Ala Ile Lys Lys Ile Asn Asp Val Phe
 35 40 45
 65 Glu His Val Ser Asp Ala Thr Arg Ile Leu Arg Glu Ile Lys Leu Leu
 50 55 60
 Arg Leu Leu Arg His Pro Asp Ile Ala Glu Ile Lys His Ile Met Leu
 65 70 75 80
 70 Pro Pro Ser Arg Arg Glu Phe Gln Asp Ile Tyr Val Val Phe Glu Leu
 85 90 95

	Met	Glu	Ser	Asp	Leu	His	Gln	Val	Ile	Arg	Ala	Asn	Asp	Asp	Leu	Thr	
				100					105						110		
5	Pro	Glu	His	Tyr	Gln	Phe	Phe	Leu	Tyr	Gln	Leu	Leu	Arg	Ala	Leu	Lys	
			115					120					125				
	Tyr	Ile	His	Ala	Ala	Asn	Val	Phe	His	Arg	Asp	Leu	Lys	Pro	Lys	Asn	
		130					135					140					
10	Ile	Leu	Ala	Asn	Ser	Asp	Cys	Lys	Leu	Lys	Ile	Cys	Asp	Phe	Gly	Leu	
	145					150					155					160	
	Ala	Arg	Ala	Ser	Phe	Asn	Asp	Ala	Pro	Ser	Ala	Ile	Phe	Trp	Thr	Asp	
15					165					170						175	
	Tyr	Val	Ala	Thr	Arg	Trp	Tyr	Arg	Ala	Pro	Glu	Leu	Cys	Gly	Ser	Phe	
				180					185					190			
20	Phe	Ser	Lys	Tyr	Thr	Pro	Ala	Ile	Asp	Ile	Trp	Ser	Ile	Gly	Cys	Ile	
			195					200					205				
	Phe	Ala	Glu	Leu	Leu	Thr	Gly	Arg	Pro	Leu	Phe	Pro	Gly	Lys	Asn	Val	
		210					215					220					
25	Val	His	Gln	Leu	Asp	Ile	Ile	Thr	Asp	Leu	Leu	Gly	Thr	Pro	Ser	Ser	
	225					230					235					240	
	Glu	Thr	Leu	Ser	Arg	Ile	Arg	Asn	Glu	Lys	Ala	Arg	Arg	Tyr	Leu	Ser	
30					245					250					255		
	Thr	Met	Arg	Lys	Lys	His	Ala	Val	Pro	Phe	Ser	Gln	Lys	Phe	Arg	Asn	
				260					265					270			
35	Thr	Asp	Pro	Leu	Ala	Leu	Arg	Leu	Leu	Glu	Arg	Leu	Leu	Ala	Phe	Asp	
			275					280					285				
	Pro	Lys	Asp	Arg	Pro	Ser	Ala	Glu	Glu	Ala	Leu	Ala	Asp	Pro	Tyr	Phe	
		290					295					300					
40	Ala	Ser	Leu	Ala	Asn	Val	Glu	Arg	Glu	Pro	Ser	Arg	His	Pro	Ile	Ser	
	305					310					315					320	
	Lys	Leu	Glu	Phe	Glu	Phe	Glu	Arg	Arg	Lys	Leu	Thr	Lys	Asp	Asp	Val	
45					325					330					335		
	Arg	Glu	Leu	Ile	Tyr	Arg	Glu	Ile	Leu	Glu	Tyr	His	Pro	Gln	Met	Leu	
				340					345					350			
50	Gln	Glu	Tyr	Met	Lys	Gly	Gly	Glu	Gln	Ile	Ser	Phe	Leu	Tyr	Pro	Ser	
			355					360					365				
	Gly	Val	Asp	Arg	Phe	Lys	Arg	Gln	Phe	Ala	His	Leu	Glu	Glu	Asn	Tyr	
		370					375					380					
55	Ser	Lys	Gly	Glu	Arg	Gly	Ser	Pro	Leu	Gln	Arg	Lys	His	Ala	Ser	Leu	
	385					390					395					400	
	Pro	Arg	Glu	Arg	Val	Gly	Val	Ser	Lys	Asp	Gly	Tyr	Asn	Gln	Gln	Asn	
60					405					410					415		
	Thr	Asn	Asp	Gln	Glu	Arg	Ser	Ala	Asp	Ser	Val	Ala	Arg	Thr	Thr	Val	
				420					425					430			
65	Ser	Pro	Pro	Met	Ser	Gln	Asp	Ala	Gln	Gln	His	Gly	Ser	Ala	Gly	Gln	
			435					440					445				
	Asn	Gly	Val	Thr	Ser	Thr	Asp	Leu	Ser	Ser	Arg	Ser	Tyr	Leu	Lys	Ser	
		450					455					460					
70	Ala	Ser	Ile	Ser	Ala	Ser	Lys	Cys	Val	Ala	Val	Lys	Asp	Asn	Lys	Glu	
	465					470					475					480	

Pro Glu Asp Asp Tyr Ile Ser Glu Glu Met Glu Gly Ser Val Asp Gly
485 490 495
5 Leu Phe Glu Gln Val Phe Arg Met Gln Phe Leu Val His Asn Asp Asp
500 505 510
Asp Asp Gln Cys Lys Ile Leu
515

10 (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
15 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

 (ii) MOLECULE TYPE: other nucleic acid
20 (A) DESCRIPTION: /desc = "Synthetic DNA"

 (iii) HYPOTHETICAL: NO

 (iv) ANTI-SENSE: NO

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

30 AAAAGCACAA GTTGCTGC

18

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
35 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "Synthetic DNA"

 (iii) HYPOTHETICAL: NO

45 (iv) ANTI-SENSE: NO

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TAACGTCTAT CGACTTCT

18

(2) INFORMATION FOR SEQ ID NO:5:

- 55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 25 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
60 (D) TOPOLOGY: linear

 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "Synthetic DNA"

65 (iii) HYPOTHETICAL: NO

 (iv) ANTI-SENSE: NO

70

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AACACAGTGG AAATGGAGTT CTTCA

25

(2) INFORMATION FOR SEQ ID NO:6:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1678 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
10 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

15 (iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Oryza sativa*

20 (B) STRAIN: C101A51

(vii) IMMEDIATE SOURCE:

(B) CLONE: BIMK1

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GTAATTTT	CCCCATCACC	ACCACCACCA	CCATCGCTTT	CTTCATCTTC	GCCTTCTGGT	60
30	CTGCATCCAT	CCATCCATCC	ATTACTCGCC	GAAGACTTCG	CGCGGGGAGA	120
	GCTTTGTCGC	GGGAACGCGG	GAAGAAAGGT	CCGAGCTTGG	AAGGAGAAGA	180
	AAGAACGCGA	GAGCTTGGA	GGCGAGCTGC	GGTGGTGTAG	CTAGCCAATG	240
35	ACTGGTAGGG	AGGGGGATGG	GGGGAGGGGG	CACGCTCGTC	GACGGATTCC	300
	CCACCGCCGC	ACGGCGTCCG	GCTCCAACCA	GTCGTCCAAC	GCCGGCGAGG	360
	CTCCGACCTC	GAGGTCGCCG	ACGACCCGGA	TCTCGTCGCC	CTCCGCTCCA	420
40	CGTGCCCAAG	CGCAAGATGC	CCTCTCCCCG	TCGAGAGCCA	CAAGAAGGTG	480
	TAGTGTGAAG	TGTGCTTTGC	TTTGCTTCGT	TTTCTTTTCA	GTTTGGGGGT	540
45	TTTCAGGCTT	TCTCGTGATC	CCTTGATTCTG	TGGCCACGAG	GGGTTCTTAG	600
	ATCTTCGTGG	CACCTGAATT	ACTTGCATAC	TGTACAATAT	CATTATTTCT	660
	GATCTGTGCA	AAGTGCAATA	CAGCTCAAGT	GCAGGTAAAG	CTTGCGTGTT	720
50	TTTCTTCTTT	CGATGGTTGC	TTGTAGAGCG	ATAGTTGCTT	GTAAACTGCC	780
	TCATCCTGGC	TGTTGACCTG	GTTATTCCAG	TGATCTATGA	AACGATCGAT	840
55	TTAAGTTTCT	TTCTTTCTTT	CTCTGTTTGC	TTAGTTCTGA	AATTACTTGC	900
	TCCATTTCTT	AGAGGAGTGC	AATTGCAGCA	CTACTATGCA	AAAAGCTTGT	960
60	GGAGCTGTTC	TCAACAATTG	GTCCCATCAT	CCTGTCATAC	TGATCCTTAG	1020
	CAAGTTGTCC	ACTTGTGGTT	TTGGATGATC	TCATCAGAAT	CGGCTACTAA	1080
	AGGTTTGACT	TGGTCTGGCC	TATGTATATC	TCTGGTACGG	ACTGTTTCTA	1140
65	GTCGCGTCTT	GCACATGGTA	TGGAGCAGGT	GTCCTTTCAT	TTGCGAATAA	1200
	CTATGTACTG	AAAATGTCAA	TTTTTGTTAG	GTTGGTCAATG	ATATTCCCAG	1260
70	ATGGTTTTGT	TTATAGGGCT	ATTCTACTAC	TGAAGAAGTT	TTATAACCAG	1320
	TTTTTAGTTA	GCTTAATATT	TTCTTGCAAA	ATTGTGATCT	TGTAGAACAC	1380
				AGTGGAAATG		

	GAGTTCTTCA	CTGAGTATGG	AGAAGCAAGC	CAGTACAGCC	AGTACCAGAT	CCAGGAAGTC	1440
	ATTGGCAAAG	GAAGTTATGG	AGTAGTTGCT	GCTGCAGTAG	ATACCCGCAC	GGGTGAGCGG	1500
5	GTTGCGATCA	AGAAATCAAT	GATGTGTTTG	AGCATGTATC	AGACGCTACG	CGCATATTGC	1560
	GTGAGATCAA	GCTCCTTCGT	CTGCTCCGTC	ACCCAGACAT	AGCTGAGATC	AAACACATTA	1620
	TGCTTCCCCC	TTCTCGAAGG	GAGTTCCAAG	ATATTTATGT	TGTTTTTGAG	CTCATGGAA	1679

Claims:

1. An isolated deoxyribonucleic acid comprising a nucleic acid sequence that encodes a protein encoded substantially by the sequence from about position 13 through about position 1569 of SEQ ID NO.:1.
2. The deoxyribonucleic acid of claim 1 operably linked to a plant-active promoter.
3. An expression vector capable of transforming a plant cell which contains the deoxyribonucleic acid of claim 1 operably linked to a promoter that is active in said plant.
4. A plant cell transformed with the vector of claim 3.
5. A plant containing a plant cell of claim 4.
6. A seed of the plant of claim 5.
7. The expression vector of claim 3, wherein the plant is a monocot.
8. The plant cell of claim 4, wherein the plant is a monocot.
9. The plant of claim 5, wherein the plant is a monocot.
10. The seed of claim 6, wherein the plant is a monocot.
11. The plant cell of claim 4, wherein the plant is rice, wheat, maize, barley or asparagus.
12. The plant of claim 5, wherein the plant is rice, wheat, maize, barley or asparagus.
13. The plant of claim 5, wherein the plant is rice.
14. A seed of the plant of claim 12 or 13.
15. The deoxyribonucleic acid comprising the sequence from about position 13 through about position 1569 of SEQ. ID. NO.:1.
16. Isolated messenger RNA complementary to the deoxyribonucleic acid of claim 1 or 15.

17. A deoxyribonucleic acid molecule or a ribonucleic acid molecule that hybridizes to the deoxyribonucleic acid of claim 1 or 15 or its complement under stringent hybridization conditions.

5 18. An isolated protein comprising substantially the amino acid sequence of SEQ ID. NO.:2.

19. A method for conferring disease resistance to a plant which comprises genetically modifying the plant to cause or regulate the expression of the
10 deoxyribonucleic acid of claim 1 or 15.

20. The method of claim 19, wherein the plant is a monocot.

21. The method of claim 19, wherein the plant is rice, wheat, maize, barley or asparagus.

15 22. The method of claim 21, wherein the plant is rice.

23. A plant promoter having a nucleotide sequence substantially contained in SEQ ID NO:6.

1/2

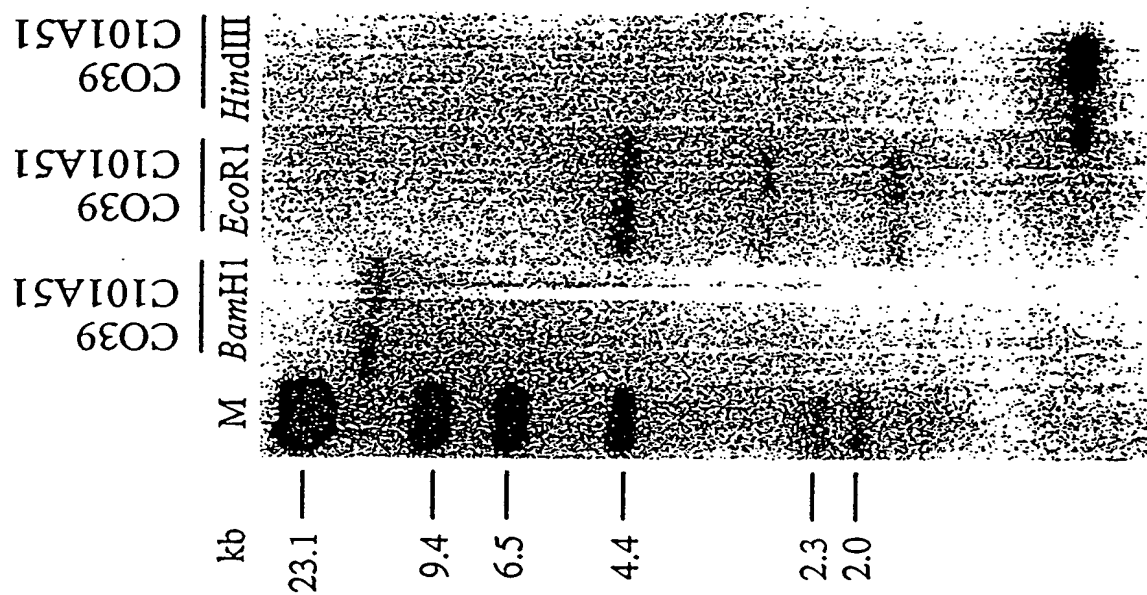


Figure .1

2/2

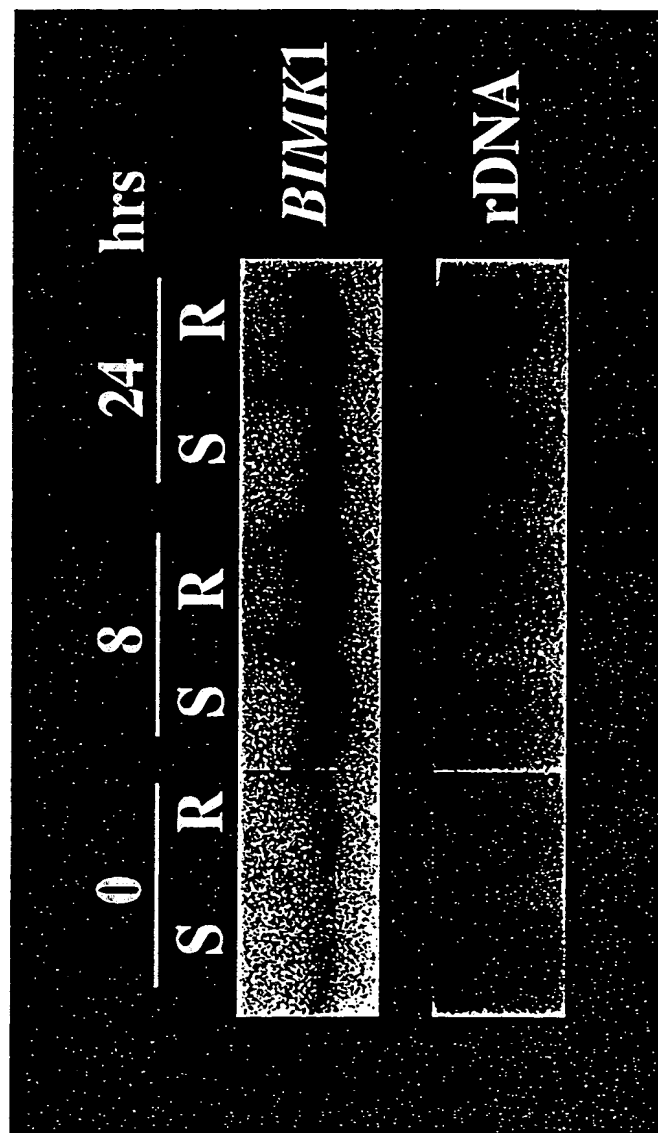


Figure 2

INTERNATIONAL SEARCH REPORT

Internat. Application No.

PCT/SG 98/00004

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/29 C12N15/54 C07K14/415 C12N9/12 A01H5/00
A01N65/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N A01H A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHOI, D.W., ET AL.: "Selaginella lepidophylla MAP kinase-like protein (sdhn-6r) mRNA, partial cds" EMBL SEQUENCE ACCESSION NO. U96716, 23 May 1997, XP002079536 see the whole document --- -/--	17

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

Date of the actual completion of the international search

6 October 1998

Date of mailing of the international search report

15/10/1998

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	<p>DATABASE WPI Section Ch, Week 9720 Derwent Publications Ltd., London, GB; Class B04, AN 97-220416 XP002079552 & JP 09 065881 A (NORINSUISANSHO NOGYO SEIBUTSU SHIGEN), 11 March 1997</p>	17
A	<p>see abstract -& "MAP kinase #1" GENESEQ ACCESSION NO. W15512, 4 June 1997, XP002079540 see the whole document -& "MAP kinase #1 coding sequence" GENESEQ ACCESSION NO. T60349, XP002079541 see the whole document ---</p>	2,3,19
X	<p>SEO, S., ET AL.: "Tobacco MAP kinase: a possible mediator in wound signal transduction pathways" SCIENCE, vol. 270, 22 December 1995, pages 1988-1992, XP002079542</p>	17
A	<p>see the whole document -& OHASHI, Y., ET AL.: "Tobacco mRNA for WPIK, complete cds" EMBL SEQUENCE ACCESSION NO. D61377, 26 December 1995, XP002079543 see the whole document ---</p>	2,3
A	<p>RONALD, P.C., ET AL.: "The molecular basis of disease resistance in rice" PLANT MOLECULAR BIOLOGY, vol. 35, September 1997, pages 179-186, XP002079544 see page 181, left-hand column, last paragraph - page 183, left-hand column, paragraph 1 ---</p>	1-23
	<p>---</p> <p style="text-align: center;">-/--</p>	

INTERNATIONAL SEARCH REPORT

Internat I Application No

PCT/SG 98/00004

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FR 2 708 613 A (NAT INST AGROBIOLOGICA ; JAPAN RES DEV CORP) 10 February 1995 see the whole document ---	1-23
A	FR 2 708 614 A (NAT INST AGROBIOLOGICA ; RESEARCH DEV CORP) 10 February 1995 see the whole document ---	1-23
A	WANG G -L ET AL.: "RFLP MAPPING OF GENES CONFERRING COMPLETE AND PARTIAL RESISTANCE TOBLAST IN A DURABLY RESISTANT RICE CULTIVAR" GENETICS, vol. 136, April 1994, pages 1421-1434, XP002063583 see the whole document ---	1-23
A	KAHMANN, R., ET AL.: "SIGNALING AND DEVELOPMENT IN PATHOGENIC FUNGI - NEW STRATEGIES FOR PLANT PROTECTION" TRENDS IN PLANT SCIENCE, vol. 2, no. 10, October 1997, pages 366-368, XP002079545 see the whole document ---	1-23
A	ZHANG, S., ET AL.: "Salicylic acid activates a 48-kD MAP kinase in tobacco" THE PLANT CELL, vol. 9, May 1997, pages 809-824, XP002079546 see the whole document -& "Nicotiana tabacum salicylic acid-activated MAP kinase (NtSIPK)" EMBL SEQUENCE ACCESSION NO. U94192, 2 June 1997, XP002079547 see the whole document ---	1
A	LIGTERINK, W., ET AL.: "Receptor-mediated activation of a MAP kinase in pathogen defense of plants" SCIENCE, vol. 276, 27 June 1997, pages 2054-2057, XP002079548 see the whole document -& LIGTERINK, W., ET AL.: "Petroselinum crispum mRNA for MAP kinase I" EMBL SEQUENCE ACCESSION NO. Y12785, XP002079549 see the whole document ---	1
A	SASAKI, T., ET AL.: "Rice cDNA, partial sequence (C11824_2A)" EMBL SEQUENCE ACCESSION NO. C22363, 15 August 1997, XP002079550 see the whole document ---	1

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INTERNATIONAL SEARCH REPORT

Intern al Application No

PCT/SG 98/00004

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>FENG, J., ET AL.: "F8N4-Sp6 IGF Arabidopsis thaliana genomic clone F8N4" EMBL SEQUENCE ACCESSION NO. B18817, 12 September 1997, XP002079551 see the whole document -----</p>	23

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern: 11 Application No

PCT/SG 98/00004

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR 2708613 A	10-02-1995	AU 6879994 A	16-02-1995
		AU 7318198 A	20-08-1998
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		JP 7163371 A	27-06-1995
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		CN 1104252 A	28-06-1995
		JP 7163357 A	27-06-1995

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